

## **APPENDIX 4**

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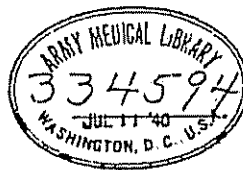
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## EXPERIMENTAL INTERSEXUALITY

### THE PARADOXICAL EFFECTS OF ESTROGENS ON THE SEXUAL DEVELOPMENT OF THE FEMALE RAT<sup>1</sup>

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#### EIGHT FIGURES

The feminizing effects of estrogens on the sexual differentiation of genetic male rats has been reported (Greene, Burrill and Ivy, '38 a, '39 a). Estradiol and estradiol dipropionate were given to pregnant rats. The male offspring were feminized in that development of the epididymis, vas deferens and seminal vesicle was inhibited and prostates were absent; a vagina, parts of the uteri and nipples were present.

In the earlier report it was stated that the females of these litters showed some gross changes from the normal. These changes were: precocious development of nipples, gross enlargement of the uteri and inhibition of the ovarian capsule. Since this report, twenty-one of these females have been serially sectioned and studied in detail. The findings are paradoxical in that the development of certain female structures is inhibited and certain structures characteristic of the male are present.

#### PROCEDURE

The estradiol and estradiol dipropionate<sup>2</sup> were given subcutaneously in oil solution to the pregnant rats. The total dosages used and the periods of pregnancy during which

<sup>1</sup> Supported in part by a grant from the Josiah Macy Jr. Foundation.

<sup>2</sup> The alpha estradiol and estradiol dipropionate were generously supplied by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products.

TABLE I

LITTER NO.	TOTAL DOSAGE	DAYS OF TREATMENT	STATUS OF WOLFFIAN DUCTS R	L	BIFURCATION OF VAGINA	SEM. VES. ANSAGE
Alpha estradiol						
79-C	0.8 mg.	13-20	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.		Abs.
291-B	7.5	13-20	Not pres	Almost comp. Part. pat.	Bifurcated	Pres. L
286-B	10.0	13-17	Comp. and pat.	Comp. and pat.	Bifurcated	Pres. R
Estradiol dipropionate						
192-A	2.0	14	Almost comp. Part. pat.	Intermittent	Bifurcated	Pres. R
202	3.25	13-17	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.	Not bifurcated	Abs.
225-A	3.0	13-17	Ej. duct remn. (fused with vagina)	Ej. duct remn. (fused with vagina)	Not bifurcated	Abs.
230-A	7.0	12-16	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.	Not bifurcated	Abs.
246-A	10.0	13-18	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.	Not bifurcated	?
262-B <sup>1</sup>	10.0	13-17	Ej. duct remn.	Ej. duct remn.	Not bifurcated	Abs.
254	13.0	13-17	Almost comp. Patent	Cranial remn. Ej. duct remn.	Bifurcated	Abs.
257-B	20.0	13-19	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.	Bifurcated	Abs.
276-A <sup>1</sup>	20.0	13-17	Ej. duct remn.	Almost comp. Part. patent.	Bifurcated	Abs.
288-C <sup>2</sup>	20.0	13-19	Cranial remn.	Cranial remn. Lower half pres. and patent	Not bifurcated	Abs.
271	22.0	13-21	Not pres.	Not pres.	Not bifurcated	Abs.
265-A	25.0	13-19	Intermittent	Intermittent	Bifurcated	Abs.
266-A	25.0	13-19	Comp. and pat.	Intermittent (part pat.)	Bifurcated	Pres. R
272-A	35.0	13-17	Cranial remn. Ej. duct remn.	Almost comp. Part. patent	Bifurcated	Pres. R
277-A	50.0	13-17	Almost comp. Part. patent.	Intermittent	Bifurcated	Abs.
279-A	60.0	13-17	Cranial remn. Ej. duct remn.	Almost comp. Part. patent	Not bifurcated	Abs.
280-A	50.0	13-17	Cranial remn. Ej. duct remn.	Cranial remn. Ej. duct remn.	Bifurcated	Abs.
284-A	100.0	13	Intermittent Part. patent	Intermittent Part. patent		Abs.

<sup>1</sup> Mother bilaterally adrenalectomized on thirteenth day of pregnancy.<sup>2</sup> Mother unilaterally castrated on thirteenth day of pregnancy.

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treatment was administered are tabulated (table 1). The estradiol dipropionate was administered in both single and divided doses, the estradiol in divided doses only. The effects of treatment on the mother and the percentage of animals carrying to term will be reported later.

After delivery by cesarian section on the twenty-second day of pregnancy (the usual time of parturition in our rat colony), at least one male and one female from each litter were killed and examined under a dissecting microscope. Since the males and females of the higher dosage litters are externally identical it was sometimes necessary to sacrifice several animals in order to obtain one of each sex. The remaining young were given to foster mothers.

## OBSERVATIONS

Stimulation of certain normal female structures has been found in all of these females. Normally nipples appear about the fourth day postpartum in the female rats of our colony, but in the modified females large, well-developed nipples are present at birth. In the normal newborn the uteri are thin, thread-like structures extending from the ovaries at the caudal base of the kidney to behind the base of the bladder where they fuse to form the vagina (fig. 1). In the modified females the uteri are grossly thickened, the diameter being two to three times greater than normal (fig. 2). Microscopic examination and measurements definitely prove that this enlargement is due to actual growth as well as to distention by secreted fluid. There is also some enlargement of that portion of the vagina which is present (figs. 3 and 4).

Inhibition of normal female structures also occurs in these modified females. Normally the ovary of the newborn female is almost completely covered by the ovarian capsule. In the modified animals the ovarian capsule is lacking and consequently the gonads are bare. This absence of the ovarian capsule is also found in the masculinized females produced by giving large doses of androgens to the pregnant rat (Greene, Burrill and Ivy, '38 b, '39 b). Development of the lower



vagina is also inhibited in the modified females. Normally longitudinal fission of the urogenital sinus into urethra and lower vagina is almost complete at birth, only the caudal part of the vagina remains attached to the urethra by a

## ABBREVIATIONS

B., bifurcation of upper vagina  
E.D., ejaculatory duct  
EF., efferent tubules  
O., ovary  
OV., oviduct

S.V., seminal vesicle  
U., urethra  
UT., uterus  
V., vagina  
W.D., wolffian duct

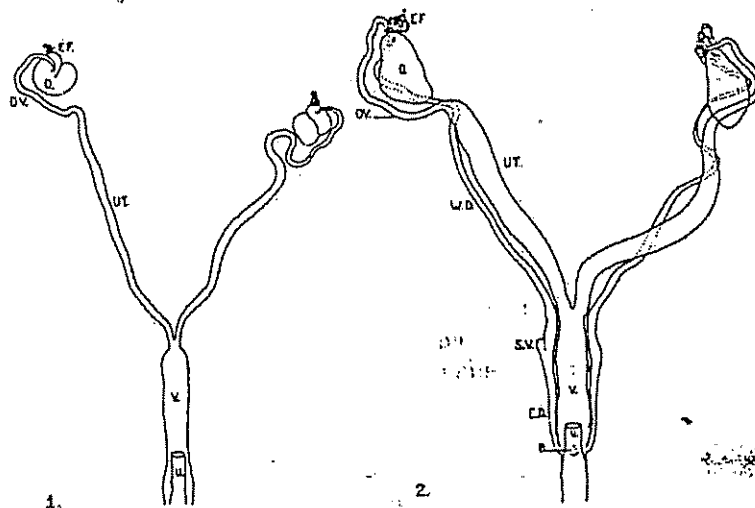


Fig. 1 Normal newborn female. Sketch made from graphic reconstruction.

Fig. 2 286-B. Newborn female. Mother received 10 mg. of estradiol during the thirteenth to seventeenth days of pregnancy.

bridge of epithelial cells. In the experimental animals the fission process has been inhibited so that the lower vagina is only partially separated from the urethra. In some cases the extreme caudal part of the lower vagina is not formed at all. This inhibition of vaginal development is similar to, but less extensive than that found in the masculinized females obtained by giving androgens to the pregnant rat (Greene,

Fig. 3 266-A. Accompanying photo on thirteenth to sixteenth days of pregnancy.

Fig. 4 Normal



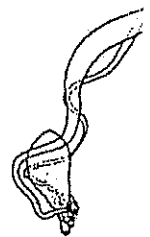
Fig. 3 266-A. Newborn female. Section through upper vagina showing accompanying ejaculatory ducts. Mother received 25 mg. estradiol dipropionate on thirteenth to nineteenth days of pregnancy. X 75.

Fig. 4 Normal newborn female. Section through upper vagina. X 75.

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Burrill and Ivy, '38 b, '39 b). Another abnormality in vaginal development has been noted in some of the modified females. At the level where the upper vagina is continuous with the lower, the upper vagina is bifurcated. This condition probably represents an inhibition or arrest of normal development. Normally the upper vagina is formed by the fusion of the müllerian ducts. The extreme caudal portions of the ducts, where they join the urogenital sinus, remain separate as late as the twenty-first day. Between the twenty-first and twenty-second days they fuse so that at birth the transition point between the upper and lower vagina is not apparent. This final fusion has failed to occur at the proper time in the modified females so that the caudal end of the upper vagina is bifurcated (fig. 2). Whether or not this bifurcation disappears after birth is not yet known.

Besides these effects, both stimulatory and inhibitory, on various female structures, some stimulation of male structures is evident in the modified females. Normally, in the female, the wolffian duct starts to regress on the seventeenth day of development and continues on subsequent days until, on the twenty-first day, only two remnants remain. One remnant, usually very small, is situated cranially and is continuous with the efferent tubules; the other is located caudally and represents the homologue of the male ejaculatory duct. This caudal remnant ordinarily becomes involved in the formation of a small portion of the vagina and is not present as a discrete structure at birth. In the modified newborn females, wolffian ducts have been found in various states of preservation. In some, only the caudal remnants are preserved as

Fig. 5 Normal newborn female. Section of uterus.  $\times 75$ .

Fig. 6 266-A. Newborn female. Section of uterus and accompanying wolffian duct.  $\times 75$ .

Fig. 7 266-A. Newborn female. Section through uteri. Right wolffian duct is well developed and patent, left wolffian duct non-patent. Mother received 25 mg. estradiol dipropionate on thirteenth to seventeenth days of pregnancy.  $\times 75$ .

Fig. 8 266-B. Newborn female. Same region as shown in figure 7. Both wolffian ducts well developed and patent. Mother received 10 mg. estradiol on thirteenth to seventeenth days of pregnancy.  $\times 75$ .

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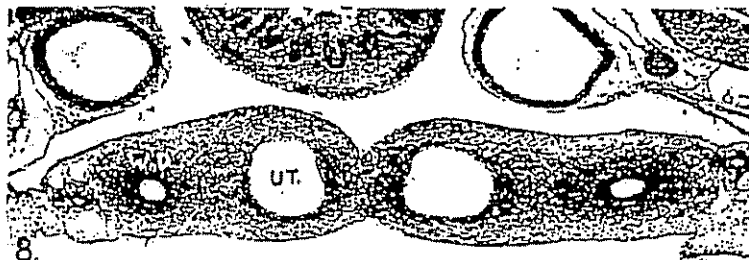
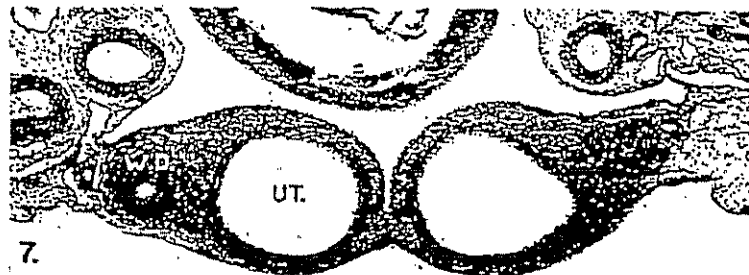
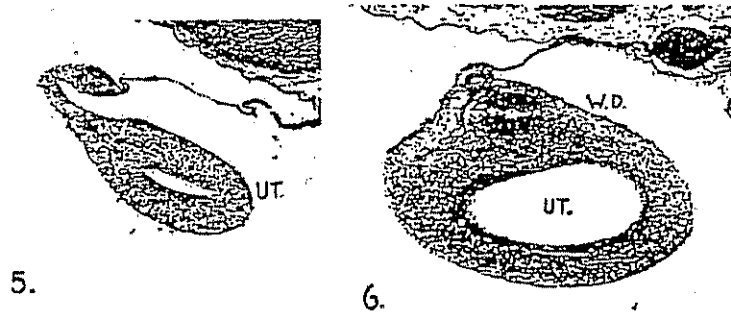
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short, solid epithelial cords which fuse with the vaginal epithelium. This condition represents the lowest degree of retention noted. More and more complete preservation of the wolffian ducts is found, culminating in complete retention of both wolffian ducts as well-developed patent structures extending from the efferent tubules, with which they are continuous, to the vagina with which they also communicate (fig. 2). True seminal vesicles have not been found in these animals, but in five animals a partial constriction of the vas has been noted at the level where the seminal vesicles normally bud off from the wolffian duct (fig. 2).

Within the dosage range which we have used to date (0.8 to 100 mg.) there has been no evidence of a correlation between the amount of estrogen administered to the pregnant rat and the degree of effect on the female offspring. This lack of correlation is somewhat surprising in view of the fact that the degree of masculinization of female offspring obtained by the administration of androgens to the pregnant rat varies directly with the dosage (Burrill, Greene and Ivy, '39). However it is possible that when the maximum dosage of estrogens is increased beyond the present limit of 100 mg., such a correlation will become evident.

#### DISCUSSION

It may be argued that these changes in the females represent only arrested development and not true masculinization. The presence of the wolffian duct may be interpreted in this way since this duct is present in one period of normal female development. In these females its subsequent involution has been inhibited. Also, the fact that the wolffian duct is not highly differentiated in these females lends credence to this interpretation, i.e., the duct has been retained, but has not been stimulated to develop in a typically masculine manner. However, the tendency toward seminal vesicle formation found in five out of twenty-one of these females cannot be attributed to developmental arrest inasmuch as no seminal vesicular anlage occur in normal female development (Greene,

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Burrill and Ivy, '39 b). The females obtained from mothers which were treated with high dosages of estrogens during pregnancy may be considered as having been 'masculinized' in that the development of certain female structures has been inhibited and certain structures characteristic of the male have been caused to persist or to develop.

The behavior of the urogenital sinus in both the male and the female offspring of estrogen treated mothers is also inexplicable. In the female, normal development is partly suppressed so that an incomplete, poorly formed lower vagina is produced. As has been mentioned in a previous publication (Greene, Burrill and Ivy, '39 b), a similar arrest of urogenital sinus development may be caused by injecting estrogens directly into the normal newborn female rat. No evidence of masculine development of the urogenital sinus, i.e., the formation of prostates, has been found in these females. In the male offspring of estrogen treated mothers, masculine development of the urogenital sinus is inhibited, i.e., prostates are lacking, but there is a certain degree of development in the female direction inasmuch as a partial lower vagina is formed. The end result of the alterations in the development of the urogenital sinus is practically identical in the male and female offspring.

It should be emphasized that, in the experimental procedure employed, estrogens were administered to the pregnant rat. There is no proof, therefore, that the changes in the offspring are produced by a direct action of the estrogens on the developing fetuses. Whether the action is direct or indirect the results obtained are indeed paradoxical. The wolffian ducts have been caused to persist in the female and have undergone actual degeneration and partial disappearance in the male. Feminine development of the urogenital sinus has been inhibited in the female and stimulated in the male.

## SUMMARY

Large doses (0.8 to 100 mg) of estrogens (estradiol and estradiol dipropionate) have been administered to pregnant



rats. Twenty-one newborn females have been serially sectioned and studied. In these animals there was a stimulation of certain female structures (uteri and nipples) and inhibition of other female structures (lower vagina and ovarian capsule). In twenty animals there has been partial or complete preservation of the wolffian ducts. In one animal the wolffian duct was complete on one side and in another both ducts were complete.

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